Welcome to chapter 2.
The following chapter is called “Sperm preparation for IVF and ICSI and sperm freezing”.
The author is Bart Desmet.
After this chapter, the student should be able to know the different parameters of basic semen examination, the different origins and types of sperm samples, and to understand why sperm preparation is important in assisted reproductive technology. The student should know the different techniques for sperm preparation.

The student should also be aware of how to prepare the different types of sperm samples. Another objective of this chapter is to understand how to evaluate a prepared sample and to make the right decision regarding the treatment procedure. Finally, the student should understand the importance and the technology of sperm cryopreservation.
The outline of the presentation is as follows.

First the origin of the spermatozoa will be described and the basic semen parameters of an ejaculated semen sample will be explained.

Secondly, the importance of semen preparation and different preparation techniques will be explained. Finally, the methods and procedures for different types of sperm samples will be presented, followed by the critical evaluation of the prepared fractions for IVF and ICSI. Short conclusion will summarize the first part of the chapter.

The second part of the chapter includes information on the indications on sperm freezing and the principles and techniques of sperm freezing.

Recommended reading will be referred to at the end of the chapter.
Sperm samples in an ART laboratory can be from ejaculated, epididymal, or testicular origin. Whatever the origin, spermatozoa can be used either fresh or frozen.

Most spermatozoa used for ART are from ejaculates produced by masturbation, preferably in a room close to the laboratory. Occasionally, the ejaculate can be retrograde (obtained from the urine fraction), or the sample can be retrieved in the operation room by electro-ejaculation (e.g. paralyzed patients).

In case of surgical intervention, the sperm can be extracted by an open biopsy from the testis (TESE, testicular sperm extraction) or from an epididymal aspirate (MESA, microsurgical epididymal sperm aspiration). The surgeon can decide to aspirate sperm percutaneously from the testicle (TESA/FNA) or from the epididymis (PESA). The indication for TESE (obstructive or non-obstructive azoospermia) will determine the procedure of sampling (see chapter 4).
Before starting the preparation of a semen sample, it is important to have an idea of the different parameters that are examined. Let us focus on the ejaculate first. This will be the origin of spermatozoa for IVF or ICSI in most patients. The lower reference limit for semen volume according to WHO (2010) is 1.5 ml (Fifth centile and its 95% confidence intervals). An ejaculate is composed of about 10% of cells and 90% of liquid, mainly contributed by the seminal vesicles, bulbourethral glands and the prostate. The cells are mainly spermatozoa, but round, epithelial, or red blood cells can also be observed.

The liquid portion of the ejaculate consists of two fractions, an alkaline and an acid fraction. The first fraction, having an acidic pH, contains spermatozoa, but also a white and non-viscous fluid of the prostate. This fraction contains citric acid, zinc and acid phosphatase, and also contributes factors, perhaps albumin, to stimulate sperm motility. Furthermore, the prostate contributes fibrinolytic for liquefaction of the viscous fraction of the seminal vesicles.

The second fraction, coming from the seminal vesicles is alkaline to neutralize the acid environment of the vagina and contains fructose as energy source for the spermatozoa. The main components are fructose and prostaglandins. This secretion is yellowish and viscous.
In the next slides, the three basic parameters in the examination of a sperm sample will be described. The reference limits for these parameters were provided by the World Health Organization (WHO) in 2010 and they correspond with the semen quality data of fertile men, whose partners achieved a pregnancy after 12 months of trying to conceive or less. The first important parameter is concentration, expressed as number of spermatozoa in million per ml. The lower reference limit for sperm concentration according to WHO (2010) is 15 million per milliliter, total sperm number – 39 million per ejaculate (5th centiles and their 95% confidence intervals). The concentration of spermatozoa in the ejaculate of one individual can highly fluctuate, due to medical reasons such as a certain kind of illness, fever or due to medication or environmental causes. Sperm concentration of higher than 200 million per milliliter is defined as polyzoospermia. A total sperm count in the ejaculate (volume x concentration) below the lower reference limit is defined as oligozoospermia. In cases where spermatozoa are only found after concentrating the sample, but not in fresh sperm preparations, it is defined as cryptozoospermia. When spermatozoa are absent in the ejaculate completely, it is called azoospermia.

A 100 micrometer-deep hemocytometer chamber is recommended to assess the concentration. Improved Neubauer hemocytometer is routinely used in most laboratories, as advised by the WHO (2010).
Another parameter is the qualitative and quantitative assessment of the motility of spermatozoa. Motility is expressed as the percentage of spermatozoa in different categories according to their speed. There are four categories:

- **Type A** or the fast progressive spermatozoa with a velocity of more than 25 micrometer per second (displacement of half tail length per second)
- **Type B** or slow progressive spermatozoa with a velocity between 5 and 25 micrometer per second (minimum head length per second)
- **Type C** is sluggish non-progressive motility with a velocity of less than 5 micrometer per second and
- **Type D** are immotile spermatozoa

The lower reference limit for progressive motility is 32 % and for total motility 40 % (WHO 2010, 5th centiles and their 95 % confidence intervals). When values are lower than the WHO reference value, it is defined as asthenozoospermia. In cases where no motile sperm are observed in the whole sample, it is defined as necrozoospermia.

The standard examination of the motility is a visual microscopic examination, but **Computer Assisted Sperm Analysis (CASA)** can be helpful as well. Not only velocity, but also kinetic parameters can be analyzed with this method. Motility is a crucial parameter determining fertility, subfertility or infertility. The total percentage of A+B motility is crucial for IVF and intrauterine insemination. When only type D is present, optional tests can help to select the appropriate vital spermatozoa.
The morphometrical and morphostructural assessment of spermatozoa is a third important parameter. Different staining methods such as the modified Papanicolaou staining or the Shorr staining are commonly used in the laboratories and are advised by the WHO. Also short staining methods such as Diff Quik can be used, and several commercial staining kits are available.

At the head level, aberrations in shape, size, acrosome morphology, and the presence of vacuoles are registered. Neck/midpiece defects can be a broken neck, irregular size and shape or the presence of a cytoplasmic droplet. Tails can be too short, coiled, multiple, broken, or bent.

According to the strict WHO criteria (2010), equal or more than 4% of spermatozoa should have normal forms. If the value is lower, it is defined as teratozoospermia. Globozoospermia is a defect of the spermatozoa characterized by the absence of the acrosome and the presence of only round-headed spermatozoa.
Sperm preparation is necessary because of the following reasons:

Spermatozoa should be able to fertilize the oocyte in vivo or in vitro after the ejaculation. To be able to fertilize an oocyte, sperm need to undergo the process of capacitation. This is a biochemical process at the level of the membrane through which the spermatozoa attain another motility pattern. A status of hyperactivation prepares the spermatozoa to undergo the acrosome reaction. Therefore, spermatozoa need to be separated within a limited time interval from the seminal plasma which contains de-capacitating factors. Another reason why the sample should be prepared is to isolate a subpopulation of spermatozoa with progressive motility and normal morphology. Finally, sperm is prepared in order to eliminate dead spermatozoa, contaminating cells and debris.
It is clear now that the physiology of the spermatozoa *in-vitro* and the variation in semen parameters lead us to choose the most appropriate preparation technique. Different preparation techniques are available and applied according to the quality of the sperm and the procedure of treatment (IVF or ICSI). There are different advantages and disadvantages of each method, affecting the yield of functional and successful spermatozoa.
The sperm samples can be prepared with the following techniques:

A single or double wash with culture medium followed by centrifugation of the semen is a simple and effective technique for instances of non-andrological cases. However, generation of reactive oxygen species after centrifugation of all the semen components in the pellet might be harmful for the spermatozoa.

Migration techniques such as swim up are successful for good quality samples but require a sufficient number of progressively motile spermatozoa. The centrifugation of semen on a two- or three-layer density gradient will select motile, morphologically normal spermatozoa and is an efficient technique for lower quality samples. Filtration techniques can also be applied but they are complex and rarely performed.
The single wash technique for semen was applied by Edwards (in 1969) for the first human in-vitro fertilization.

The double wash of semen has been successfully used, especially for non-andrological and tubal IVF cases in fertility units in Australia, France, and the USA. It has been observed that failed fertilization occurred after simple wash techniques in idiopathic and andrological cases, even with high concentration of motile spermatozoa. Pelleting (centrifugation and compaction) of an unselected cell population, including leucocytes and defective spermatozoa, results in reactive oxygen species generation. They affect the membrane of vital and motile spermatozoa, and thereby reduce their fertilizing potential.
Self migration techniques are dominated by methods usually called “swim up” techniques. Spermatozoa are allowed to swim up into a fraction of culture medium. This technique mimics the in vivo migration of competent spermatozoa through the cervical mucus. Preferably, unwashed or undiluted semen is placed under a fraction of medium and spermatozoa are allowed to migrate out of the seminal plasma into the medium. The advantage is an enrichment of spermatozoa with good progressive motility and very few – if any – immotile spermatozoa. Moreover, debris and other cells are almost completely excluded. After swim up, the upper part of the overlying culture medium, containing the motile cells, is removed and centrifuged. The resulting pellet is re-suspended in culture medium.

Swim up techniques are not fully standardized: variations in type and volume of overlying medium, interface area, time and temperature of incubation, and method of harvesting are possible.

The swim down technique is a variant of the self migration swim up technique. Swim down can be performed by layering semen on top of a medium fraction, or can be performed in a specially designed Tea-Jondet tube.
This picture illustrates the swim up technique. In the first test tube of the figure (left) the pellet of the washed semen is very gently covered with medium. After swim up (middle test tube), the upper part of the medium containing the highly motile spermatozoa will be aspirated, which is shown in the test tube at right side of the figure.
The principle of density gradient centrifugation is centrifugation of semen through a
gradient of the minimum of two layers of solutions with different densities
(weight/volume). The gradient is realized by layering suspensions with different
concentrations of colloidal silica particles coated with silane, which separates cells
by their density. Most widely used and simple is the two-step discontinuous density
gradient. The suspension with the highest density is placed on the bottom of the
tube, and lower density suspensions are layered upon it. A semen fraction is gently
put on top of the gradient. Spermatozoa with a density lower than the density of the
specific suspension are unable to penetrate through the suspension and are
withheld at the interface. Centrifugation of the gradient results in distribution of
spermatozoa and other components in the different suspensions or at the interface between the layers
with different densities.

Spermatozoa harvested from the bottom are highly motile and are the best fraction
for fertilization. Immotile spermatozoa, other cells and debris are retained in the low
density fraction or at the interface between the layers with different densities.
This slide illustrates the principle of density gradient centrifugation. The test tube on the left side of the figure contains two density layers, with liquefied semen gently layered on top of the gradient. After centrifugation (test tube at the right side), the interfaces containing low density spermatozoa, often being immotile or dead, and other cells are shown between the two layers. The spermatozoa with higher density, representing the highly motile sperm population will be harvested by aspiration of the centrifugation pellet.
Variations are possible: in density of the suspensions or number of density layers. In routine settings a double gradient of 90 and 45 % or 80 and 40 % is commonly used. The Percoll gradient was the gold standard for a long time, but it included a high number of endotoxins and was therefore replaced in 1996 by different alternatives, which are more suitable for human sperm.

The density gradient is an effective preparation technique for good and low quality sperm samples. A study by Sapienza (1993) revealed that there was no difference in fertilization after IVF for good-quality samples prepared with swim-up or density gradient centrifugation technique. Higher fertilization for lower quality samples after DGC:

- Lower production of reactive oxygen species
- Less chromatin defects observed

The findings have been confirmed by Jaroudi et al. (1993). If sperm is prepared on a density gradient, there is a lower production of reactive oxygen species (ROS) and there are less chromatin defects observed, as shown by Le Lannou & Blanchard (1988) and Morales et al. (1991).
Although the technique of density gradient centrifugation is the preparation procedure of choice for most of the semen samples, it may not be the appropriate method in certain cases. If semen samples contain many round cells, these cells may create a barrier impermeable to spermatozoa during centrifugation of the gradient. The result is a low recovery rate of spermatozoa. These spermatozoa may be recuperated by aspiration and washing of the interfaces between the layers.

In case of severe oligozoospermia, the few spermatozoa present in the ejaculate may not be collected from the pellet, but lost in the lower density fraction or at the interface. The spermatozoa may be recuperated by aspiration and washing of the interfaces and lower density fraction.

(Own experiences)
Fourth category of sperm preparation procedures is the filtration technique. Spermatozoa can be separated on columns of glass wool, glass beads, or cross-linked dextran gel. Gravity helps to pull progressive motile spermatozoa through the column, which are further selected by gel density filtration, or beads of different sizes.

These preparation techniques are only effective for good quality samples. Furthermore, these procedures are complex and therefore not often performed in routine ART laboratories.
In the following slides, we will present the common steps in the preparation of 10 different types of sperm samples in an IVF laboratory, depending on the origin of the sperm and its fresh or frozen status.

Let’s start with fresh ejaculates, the most common type of samples to be prepared. Preferably, the sample is collected in a room near the lab. The embryologist performs sample and patient identification. The sample is allowed to liquefy and is evaluated according to the WHO criteria. An appropriate preparation technique is performed (mostly density gradient centrifugation, swim up or double washing according to the quality) and the selected fraction is further prepared for IVF or ICSI by concentrating or diluting when appropriate. The final fraction is assessed for concentration and motility.

<table>
<thead>
<tr>
<th>Sperm preparation 1: Fresh ejaculated semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Semen collection near the laboratory</td>
</tr>
<tr>
<td>- Reception of sample and patient identification</td>
</tr>
<tr>
<td>- Liquefaction</td>
</tr>
<tr>
<td>- Evaluation of semen parameters according to WHO criteria</td>
</tr>
<tr>
<td>- Preparation technique: density gradient, swim up or double wash</td>
</tr>
<tr>
<td>- Preparation of the selected fraction for IVF or ICSI</td>
</tr>
<tr>
<td>- Concentration</td>
</tr>
<tr>
<td>- Dilution</td>
</tr>
<tr>
<td>- Evaluation of final fraction: concentration/motility</td>
</tr>
</tbody>
</table>
In patients with retrograde ejaculation, semen passes into the bladder at ejaculation. After alkalinization of the bladder by intake of sodium bicarbonate on the day before semen production, three fractions are collected. The antegrade fraction or ejaculate (fraction 1), collected by masturbation, has a small volume and mostly contains no spermatozoa. After ejaculation, the patient produces the retrograde fraction by collecting the urine. The initial urine fraction (10 ml) is collected in a cup containing 10 ml buffered medium supplemented with albumin (fraction 2). The rest of the urine sample is collected in a third sample cup (fraction 3).

The antegrade sample is examined first. If no sperm is found, the retrograde fraction (fraction 2) is washed and concentrated. When spermatozoa are present, the sample is further processed by double washing.
In exceptional cases such as for spinal cord injured or impotent patients, the sample can be retrieved by rectal electro stimulation of the accessory glands.

The antegrade fraction and retrograde fractions are collected in buffered medium with albumin. The antegrade fraction is prepared similarly as normal ejaculated sperm. The retrograde fraction is washed and concentrated.

The decision to freeze electro-ejaculated semen depends on sperm quality.
This slide presents the treatment of frozen ejaculated sperm samples. Frozen samples can be partner sperm or donor sperm. The required number of straws for thawing depends on the indication, the sperm quality, and the fertility treatment or procedure which will be applied. It is important that the identity of the male is double checked on the straws and in the database.

The straws are thawed on the bench of the laminar air flow at room temperature and are disinfected before opening with sterile scissors. From this step onwards, the preparation is similar as for fresh ejaculate samples. Take notice that in case of simple sperm washing after thawing, the medium should be added drop by drop in order to avoid an osmotic shock.
Testicular sperm can be obtained by open scrotal surgery under local or general anesthesia. The reason for the intervention can be therapeutic (with immediate use for ICSI) or diagnostic (with sperm freezing for later ICSI, if available). The biopsy samples of testicular tissue are collected in buffered medium and transported to the IVF laboratory. The samples are mechanically minced with surgical needles or scissors under the stereomicroscope in order to release the spermatozoa from the tubuli, according to Verheyen et al. 1995. Afterwards, the suspension is examined under the inverted microscope at 400x magnification. Different preparation techniques for TESE can be applied depending on the presence or absence of spermatozoa.

The indications and the practical management for testicular and epididymal sperm retrieval are described extensively in chapter 4.
When free motile spermatozoa are observed, the first step in the separation is made by allowing larger cells and tissue pieces to sediment in a conical test tube for one minute. The supernatant medium is aspirated and concentrated by centrifugation for 5 minutes at 750 times gravity. The pellet retrieved from this centrifugation step is re-suspended and droplets of 10 microliter are prepared in a Petri dish and overlaid with oil. At this time, the aspiration and isolation of spermatozoa with a micropipette can start. When only immotile spermatozoa, or spermatozoa attached to other testicular cell types or no spermatozoa at all are observed, additional preparation techniques will be applied: enzymatic digestion of the tissue and lysis of the erythrocytes.
In this slide we describe the use of the enzymes and the lysis buffer for testicular sperm preparation. Enzymatic digestion of testicular tissue may be used if no spermatozoa, only immotile spermatozoa or only spermatozoa attached to other cell types are observed in the suspension prepared by mechanical methods.

The enzyme most commonly described in literature is collagenase I or IV. The collagenase solution is prepared in a concentration of 1,000 IU/ml. The tissue suspension is first centrifuged at 1,000xg for 5 minutes. The pellet is re-suspended in the enzyme solution and incubated in a stirring water bath at 37°C for one hour. The reaction is stopped by washing with an excess volume of buffered medium. Undissolved pieces of tissue and large cells are removed by centrifugation at 50xg for 3 minutes. The supernatant is further processed and centrifuged at 1,000xg for 5 minutes. The re-suspended pellet is used to make droplets for ICSI or can be further processed with an erythrocyte-lysing buffer in case when many red blood cells are present.

Processing with erythrocyte-lysing buffer can be applied after enzymatic digestion of testicular tissue in cases when many red blood cells are present in the sample and hinder visualization of spermatozoa.

The buffer is prepared according to Verheyen et al. (1997). The pellet of the digested tissue with concentrated red blood cells is incubated in 2 to 3 ml erythrocyte-lysing buffer at room temperature for 5 minutes. Afterwards, the buffer is washed out with an excess volume of buffered medium at 750xg for 5 minutes. Droplets of 10 microliter under oil are prepared and the aspiration and isolation of spermatozoa can begin at this point.
A fine needle aspiration is a non-invasive procedure which is applied in case of obstructive azoospermia or more rarely in case of a problem to produce the ejaculate. Petri dishes with 10 microliter droplets of buffered medium covered with oil are prepared. Aspirations from the testis are released into the droplets and checked for motile sperm in the laboratory. Repeated aspirations can be performed in order to obtain enough motile spermatozoa for ICSI. With this technique, no freezing for future ICSI use will be possible because of the small aspirated volumes released under oil.

For more details on this topic, please see chapter 4.
In case of obstructive azoospermia, sperm can also be recovered by microsurgery at the level of the epididymis, with the so called MESA or microsurgical epididymal sperm aspiration.

The aspirates are released in a tube containing 0.2ml of buffered medium. If the sample is of sufficient quality, density gradient centrifugation may be applied in order to prepare the fractions for ICSI. In most cases, many motile spermatozoa are retrieved and MESA samples can be frozen for future use.

When sperm is obtained by a percutaneous fine needle aspiration, the procedure is called PESA (percutaneous epididymal sperm aspiration). The procedure to collect and to prepare PESA sperm is similar to the MESA approach.
Sperm samples retrieved from open TESE, MESA or PESA can often be frozen depending on the quality of the retrieved fractions for future use. The straws with frozen testicular sperm may contain tissue suspension or concentrated fractions obtained after mechanical treatment or after enzymatic treatment of the biopsy samples.

The most appropriate technique to prepare frozen-thawed TESE sperm is the double wash procedure. In order to avoid osmotic shock, buffered medium is added drop by drop while shaking the tube. The washing is repeated twice in order to completely remove the cryoprotectant, and centrifugation is performed at 750xg for 5 minutes. A Petri dish with 10 µl droplets under oil is prepared for ICSI. The preparation of frozen-thawed epididymal sperm is identical to the treatment of frozen-thawed ejaculates.
The embryologist should be able to critically evaluate the fractions after sperm preparation. For IVF, the final fraction preferably contains 50% rapid progressive motility spermatozoa. If lower, the risk of failed fertilization or limited fertilization rate increases according to Verheyen (1999). The sperm concentration in the final fraction should be 1 to 5 million per ml. For IVF, 5,000 spermatozoa (present in a volume of 1–5 microliter) are added to a droplet of 25 µl fertilization medium containing one or two oocytes. In case of culture in tubes, a final progressive motile sperm concentration of 0.2 to 1 million per milliliter is realized.
The final sperm fraction for ICSI preferably contains motile spermatozoa. The least sluggish motility is sufficient to discriminate between viable and dead spermatozoa. It is important to have a policy in case only immotile sperm are observed in the sample. For ejaculates, a second semen sample can be asked, which often shows better quality. If still immotile, other procedures can be applied in order to detect viable spermatozoa. The hypo-osmotic swelling test (HOS) detects viable cells if swelling at the level of the tail occurs after exposure to a hypo-osmotic medium (Verheyen et al. 1997). Laser-assisted immotile sperm selection is a more recent technique in order to select vital spermatozoa for ICSI (see chapter 3 for more information).

If the named techniques to distinguish viable and dead spermatozoa are not available, TESE (testicular sperm extraction) or FNA (fine-needle aspiration) can be applied as a back-up procedure in order to collect more freshly produced spermatozoa. Immotile spermatozoa of testicular origin have a higher chance to successfully fertilize the eggs than ejaculated immotile sperm. If frozen fractions, of whatever origin, contain only immotile spermatozoa, a fresh sample (semen or testicular) is preferably retrieved. When no spermatozoa are observed after extensive preparation of whatever type of fresh diagnostic sperm sample, the use of donor sperm may be proposed to the couple.
We can conclude that sperm samples for *in vitro* fertilization need to be processed in order to separate spermatozoa from seminal plasma and in order to select a subpopulation of motile, functionally competent spermatozoa.

Basic seminal parameters (concentration, motility) need to be assessed in order to prepare the sample for IVF or ICSI with the most appropriate technique.

The wash technique is effective in case of extreme oligozoospermia when few round cells and debris are present in the sample.
The migration technique is only effective for the preparation of good-quality semen samples with high progressive motility.

Density gradient centrifugation is the most commonly used preparation technique, suitable for high and low-quality samples for IVF and ICSI, but not in case of extreme oligozoospermia.

The prepared sperm fraction sample should be evaluated critically in order to make the right decision regarding further treatment.
The indications for cryopreservation of spermatozoa from whatever origin can be divided into three categories: medical reasons, non-medical reasons, or sperm banking.

Freezing partner sperm for medical reasons is indicated in cases of extreme oligozoospermia or cryptozoospermia, whereby the partner repeatedly provides an ejaculate sample which is frozen if motile sperm is present. Testicular or epididymal sperm can be frozen in order to avoid repeated surgeries for sperm retrieval. An important indication is fertility preservation for male patients undergoing cancer therapy, or vasectomy for sterilization.

Non-medical reasons for sperm freezing are the absence of the husband on the day of egg collection, or masturbation problems in stress situations.

Final indication for sperm freezing is sperm donation. Non-autologous donor sperm can only be released after a quarantine period and repeated serology testing of the donor in order to avoid transmission of sexually transmittable diseases.

The frozen samples for non-autologous donation should be negative for transmittable diseases such as Hepatitis B, C and HIV.
Cryopreservation of human spermatozoa dates from the late 1940s, after Polge et al. discovered the cryoprotective properties of glycerol. Addition of the cryoprotectant with high osmolarity increased the survival rate of spermatozoa stored on dry ice at -79°C. Spermatozoa are more cold-shock resistant than other cells due to their low water content and high membrane fluidity. Addition of the appropriate cryoprotectant followed by equilibration helps to minimize intracellular ice crystal formation. Spermatozoa initially shrink when the cryoprotectant is added. Equilibration returns the cell to almost its original size as the permeating cryoprotectant replaces the water inside the cell in order to obtain an osmotic equilibrium.

After thawing, the reverse process takes place by washing out the cryoprotectant. Intracellularly, the cryoprotectant is again replaced by water.

Not all spermatozoa survive the freezing procedure and cell loss is inevitable. After thawing, particularly motility of the spermatozoa is affected: about 50 % of the spermatozoa do not survive the freezing and thawing process.
Different freezing procedures are possible. Spermatozoa can be frozen either rapidly in liquid nitrogen vapor or slowly by computer controlled-rate cooling and freezing. The temperature decrease is realized in different steps by placing the sperm sample (straws) at different, decreasing levels above the surface of liquid nitrogen. A study of Verheyen et al. in 1993 revealed no difference in post-thaw sperm quality between the vapor freezing technique and the controlled-rate freezing.

Different cryoprotectants are available for freezing of biological cells, such as dimethyl sulfoxide (DMSO), ethyleneglycol, and propanediol but glycerol appears to be the best choice for spermatozoa. The cryoprotectant is added drop by drop to the sperm sample in order to reduce the osmotic shock effect on the cells. The use of extenders in the cryoprotectant solution substantially increases the survival of spermatozoa. The aim is to improve the fluidity of the sperm plasma membrane. The glycerol-egg yolk-citrate buffer is frequently used for this purpose. Several other solutions are commercially available. Different procedures and solutions are described extensively in the WHO 2010 manual.

Sperm samples may safely be frozen in high-security sealed straws. Samples are usually stored in liquid nitrogen. A switch to storage in liquid nitrogen vapor is a trend in order to avoid transmission of pathogens.
These pictures illustrate the cryopreservation of sperm samples with the related actions, tools and devices. Top left: an example of a computer controlled freezing device. Top right: storage containers and bottom right: frozen straws stored in the liquid nitrogen.
Sperm freezing technology allows patients to store sperm for medical and non-medical reasons, and is essential for the establishment of a semen donor program.

Sperm freezing is an effective technique to preserve sperm samples from ejaculated and testicular origin prior to or after an IVF or ICSI treatment. Although loss of sperm quality is inherent to sperm freezing, fast vapor freezing is an efficient technique in order to maintain acceptable quality of spermatozoa.

Glycerol (with extenders) is the common cryoprotectant for human sperm freezing. The preferred vials for storage are high-security straws.

Sperm freezing technology allows patients to store sperm for medical and non-medical reasons, and is essential for the establishment of a semen donor program.

Sperm freezing is an effective technique to preserve sperm samples from ejaculated and testicular origin prior to or after an IVF or ICSI treatment. Although loss of sperm quality is inherent to sperm freezing, fast vapor freezing is an efficient technique in maintaining acceptable post-thawing quality of spermatozoa.

Glycerol (with extenders) is the common cryoprotectant for human sperm freezing. The preferred vials for storage are high-security straws.
Recommended reading

Recommended reading


Recommended reading


Recommended reading


- Le Lannou D. and Blanchard Y. Nuclear maturity and morphology of human spermatozoa selected by Percoll density gradient centrifugation or swim-up procedures. J. Reprod. Fertil. 1988;84:551-556.

Recommended reading


Recommended reading


Recommended reading

